FluorPen FP 100

Series

Operation Manual

For: *FluorPen FP 100*

FluorPen FP 100-MAX

PAR-FluorPen FP 100-MAX-LM

FluorPen FP 100-B

FluorPen FP100-U

FluorPen or PAR-FluorPen
        with detachable leafclips

Table of Contents

1. Technical Specification

Measured and Calculated Parameters:
Fo, Ft, Fm, Fm', QY, NPQ 1*, NPQ 2*, OJIP*, LC 1*, LC 2*, PAR**

Cosine Correction:**
Cosine corrected up to 80º angle of incidence

Linearity:**
Maximum deviation of 1 % up to per 10,000 μmol s^{-1} m^{-2}

Saturating Light:
Adjustable from 0 to 3,000 μmol(photons)/m^2.s (0 to 100 %)

Actinic Light:
Adjustable from 0 to 1,000 μmol(photons)/m^2.s (0 to 100 %)

Measuring Light:
Adjustable from 0 to 3,000 μmol(photons)/m^2.s (0 to 100 %)

Detector Wavelength Range:
PIN photodiode with 697 to 750 nm bandpass filters

Communication:
Bluetooth, USB, or serial (not included in the FluorPen FP 100)

FluorPen 1.0 Software:
Windows 2000, XP, or higher compatible***

Memory Capacity:
Up to 4 Mb

Internal Data Logging:
Up to 100,000 data points

Display:
2 x 8 characters LC display

Keypad:
Sealed, 2-key tactile response

Keypad Escape Time:
Turns off after 3 minutes of no use

Power Supply:
4 AAA alkaline batteries (single use or rechargeable)

Battery Life:
48 hours typical with full operation

Low Battery Detection:
Low battery indication displayed

Size:
120 mm x 57 mm x 30 mm
4.7" x 2.2" x 1.2"

Weight:
180 g, 6.5 oz

Sample Holder:
Mechanical leaf clip (non-destructive for plants, effective for sample pre-darkening)

Operating Conditions:
Temperature: 0 to 55 ºC; 32 to 130 ºF Relative humidity: 0 to 95 % (non-condensing)

Storage Conditions:
Temperature: -10 to +60 ºC; 14 to 140 ºF Relative humidity: 0 to 95 % (non-condensing)

Warranty:
1 year parts and labor (see the last page of this Operation Manual for precise conditions)

*   Only in the FluorPen FP 100-MAX or PAR-FluorPen FP100-MAX-LM.

**   Only in the PAR-FluorPen FP100-MAX-LM.

*** Windows is a registered trademark of Microsoft Corporation.

The Bluetoothmodule BlueNiceCom III is endowed with a declaration of conformity with the following norms:

EN 300 328 V1.6.1 (2004-11)
EN 301 489-1, -3 V1.5.1 (2003-12)
EN 50371 December 2002
EN60650
FCC Part 15.247
FCC Grantee Code: R7T

Bluetooth Qualified Product Notice:
GRA_013_04
Bluetooth Listing Identifier: B01572

2. General Information

FluorPen measures the following parameters:

Ft - Instantaneous Chlorophyll Fluorescence

Ft is equivalent to Fo if the leaf sample is dark-adapted.

QY - Quantum Yield

QY is a measure of the Photosystem II efficiency. In a dark-adapted leaf this is equivalent to Fv/Fm. In a light-adapted leaf it is equivalent to Fv'/Fm'.

NPQ* - Non-Photochemical Quenching

The NPQ protocol is the most typically used measuring approach to quantify photochemical and non-photochemical quenching. The measurement should be performed with a dark-adapted sample (see more in Chapter 8.E. of this Manual).

OJIP* - Chlorophyll Fluorescence Induction Kinetics

The OJIP curves enable observing major changes that occur during exposure of plants to high irradiance (see more in Chapter 8.D. of this Manual).

Light Curve*

The LC 1 and LC 2 protocols serve to describe adaptation of Quantum Yield to six or five different light levels (see more in Chapter 8.F. of this Manual).

PAR - Photosynthetically Active Radiation**

Photosynthetically Active Radiation measured as Photosynthetic Photon Flux Density (PPFD).

* Only in the FluorPen FP 100-MAX, PAR-FluorPen FP100-MAX-LM or in the FluorPen models including the protocol update (FluorPen FP 100-B, FP 100-U, FP 100-S).

** Only in the PAR-FluorPen FP100-MAX-LM.

3. List of Equipment

Carefully unpack the carton. You should have received the following items:

- **FluorPen**
- **4 AAA Alkaline Batteries**
- **Carrying Case**
- **Textile Strap for Comfortable Wearing**
- **Self-Adhesive Rubber Pads for Optics Protection**
- **FluorPen Quick Guide**
- **This Owner's Manual** *(on a USB flash disc)*
- **Install USB flash disc with FluorPen software** *(FluorPens with communication only)*
- **Bluetooth Communication Module** *(FluorPen FP 100-B or FP 100-MAX* only)*
- **USB Communication Module** *(FluorPen FP 100-U or FP 100-MAX* only)*
- **Other Accessories or Optional Features** *(according to your specific order)*

** FluorPen FP 100-MAX includes **only one** communication module (Bluetooth or USB).*

Note:

If any item is missing, please, contact PSI. Also check the carton for any visible external damage. If you find any damage, notify the carrier and PSI immediately. The carton and all packing materials should be retained for inspection by the carrier or insurer.

For customer support, please write to: **support@psi.cz**

4. Physical Features
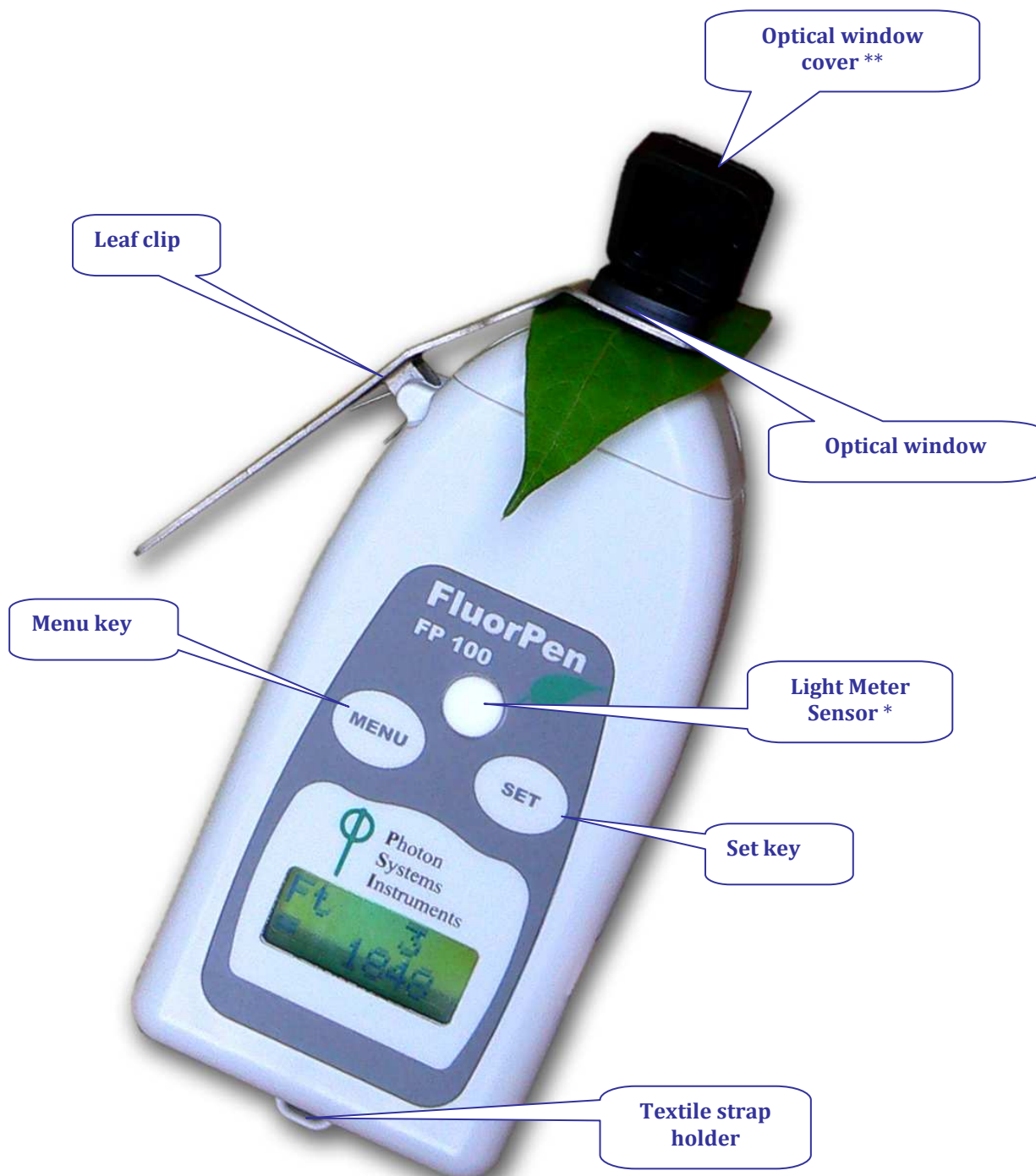
* *Only in the PAR-FluorPen FP 100-MAX-LM*
** *Only in the FluorPen FP 100-MAX-W (supplied with a special "open-window" leaf clip).*

5. Accessories

Carrying Case

The FluorPen is supplied with a carrying case which is padded to protect the instrument during transportation. You can either carry the FluorPen case over the shoulder or hang it on your belt.

Protective Rubber Pad

The optical part of the FluorPen is covered with a self-adhesive rubber pad that protects the optics from dirt or moisture. The damaged or dirty pad can be easily removed by tearing it off from the surface. To affix a new one, just remove the sheeting and stick the pad on cleaned and dried surface. Be sure that you placed the pad properly and that you did not cover the optical window.
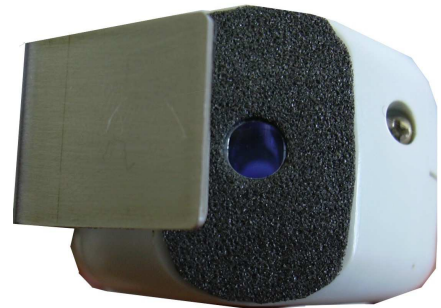
Batteries

The FluorPen operates from four AAA single-use or rechargeable batteries. They may be easily replaced by unscrewing the cover of the battery holder on the rear of the instrument. Battery life is approximately 48 hours when the FluorPen is operated continuously.

6. Operation Instructions

The next six pages explain the structure of the Main Menu and three Sub-Menus with all their options.

- The blue color represents the Main Menu and its Options.
- The yellow color represents the first-level Sub-Menus and their Options.
- The green color represents the second-level Sub-Menus and their Options.
- Full-line arrows are used for the **SET** key.   ⟶
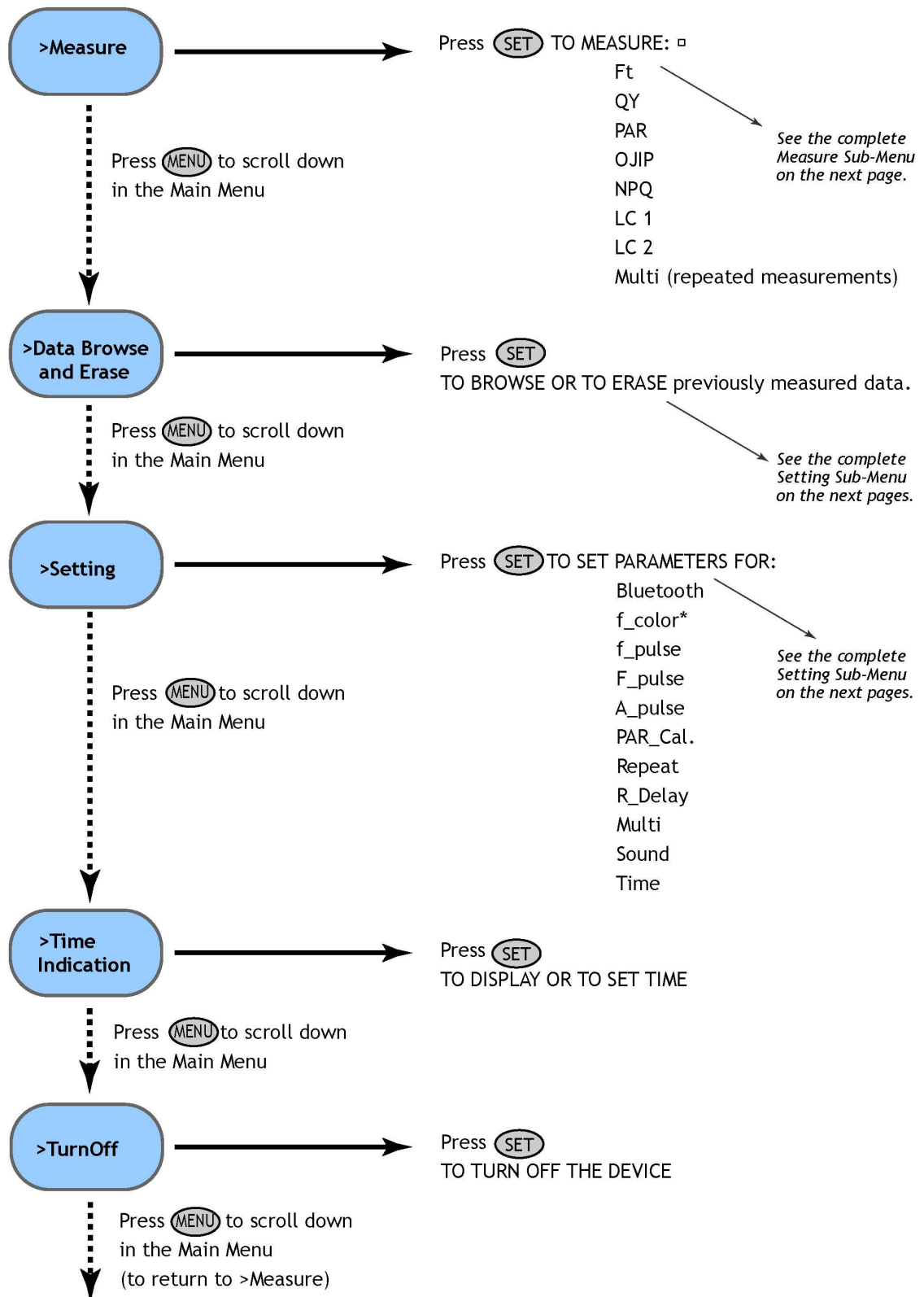- Dashed-line arrows are used for the **MENU** key.   ┈┈┈➤
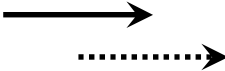
In general:
- Use the **MENU** key to scroll through sequential menu options on the digital display.
- Use the **SET** key to select a menu option based on cursor (>) position.

Main Menu

To start hold the SET key for 1 second.

>Measure

Press (SET) TO MEASURE: ▫
    Ft
    QY
    PAR
    OJIP
    NPQ
    LC 1
    LC 2
    Multi (repeated measurements)

See the complete Measure Sub-Menu on the next page.

Press (MENU) to scroll down in the Main Menu

>Data Browse and Erase

Press (SET)
TO BROWSE OR TO ERASE previously measured data.

See the complete Setting Sub-Menu on the next pages.

Press (MENU) to scroll down in the Main Menu

>Setting

Press (SET) TO SET PARAMETERS FOR:
    Bluetooth
    f_color*
    f_pulse
    F_pulse
    A_pulse
    PAR_Cal.
    Repeat
    R_Delay
    Multi
    Sound
    Time

See the complete Setting Sub-Menu on the next pages.

Press (MENU) to scroll down in the Main Menu

>Time Indication

Press (SET)
TO DISPLAY OR TO SET TIME

Press (MENU) to scroll down in the Main Menu

>TurnOff

Press (SET)
TO TURN OFF THE DEVICE

Press (MENU) to scroll down in the Main Menu
(to return to >Measure)

----
* Not active.

Measure Sub-Menu

Use the Measure Sub-Menu when measuring selected parameters.

Press **SET**
to measure:

>Measure

Press **MENU** to
scroll down in
the Main Menu

>Ft

Press **SET**
to measure Ft:

Ft = Instantaneus Chlorophyll
Fluorescence
(Ft = Fo if the sample is
dark-adapted)

>QY

Press **SET**
to measure QY:

QY = Quantum Yield
QY = Fv'/Fm' if the
sample is light-adapted)

>PAR

Press **SET**
to measure PAR:

PAR = Photosynthetically Active
Radiation measured as
Photosynthetic Photon
Flux Density

>OJIP

Press **SET**
to measure OJIP:

OJIP = Chlorophyll Fluorescence
Induction Kinetic

>NPQ

Press **SET**
to measure NPQ:

NPQ = Non-Photochemical
Quenching

>LC 1

Press **SET**
to measure LC 1:

LC 1 = Light Curve (adaptation
of Quantum Yield to 6
different light levels)

>LC 2

Press **SET**
to measure LC 2:

LC 2 = Light Curve (adaptation
of Quantum Yield to 5
different light levels)

>Multi

Press **SET** to use
the Multi Option

The Multi Option serves for
continual measurements of
a selected parameter with
a long pause (1 hour or so).
All measured values are
stored in the memory.
Learn more about this Option
in the Setting Sub-Menu graph.

Press **SET** to
return to the
Main Menu

>Return

Data Sub-Menu

Use the Data Sub-Menu when browsing or erasing previously measured data.

Press (SET) to browse
or erase saved data:

>**Data Browse
and Erase**

>**Browse**

Press (SET) to browse
previously saved data

Press (MENU) to
scroll down in
the Data Sub-Menu

Press (MENU) to
scroll down in
the Main Menu

Press (SET) to erase
previously saved data

>**Erase**

Press (SET)
once more
to confirm
your choice

Press (MENU) to
scroll down in
the Data Sub-Menu

Press (SET) to
return to the
Main Menu

>**Return**

Press (MENU) to
scroll down in
the Data Sub-Menu

IMPORTANT NOTE: Be aware that it is not possible to erase single data.
All stored data are erased!

Setting Sub-Menu - Part 1

Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.

>Setting

Press (SET) to set:

>Bluetooth

Press (SET) to turn Bluetooth on/off:

Press (SET) to confirm your selection. Press (MENU) to scroll between the BT_On and BT_Off options.

Press (MENU) to scroll down in the Main Menu

>f_color

The function is not active in the FluorPen device.

>f_pulse

Press (SET) to adjust f_pulse intensity in %:

f_pulse = Measuring Flash Pulse

Press (SET) to confirm your selection. Press (MENU) to return.

>F_pulse

Press (SET) to adjust F_pulse intensity in %:

F_pulse = Saturating Pulse

Press (SET) to confirm your selection. Press (MENU) to return.

>A_pulse

Press (SET) to adjust A_pulse intensity in %:

A_pulse = Actinic Light

Press (SET) to confirm your selection. Press (MENU) to return.

>PAR_Cal

Press (SET) to adjust PAR calibration constant:

Press (SET) to confirm your selection. Press (MENU) to return.

>Repeat

Press (SET) for the Repeat Option setting:

The Repeat Option allows for automatically repeated measurements (1 to 10 times) of a selected parameter. The average value is automatically counted and saved.

Press (SET) to confirm your selection in the Repeat Option. Press (MENU) to return to the Setting Sub-Menu.

To be continued on the next page

13

Setting Sub-Menu - Part 2

Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.

>R_Delay

Press (SET) for R_Delay setting

The R_Delay Option is used with the Repeat Option (when Repeat > 1). It sets the time interval (1 - 10 sec) between two or more measurements of a selected parameter:Ft, QY, OJIP, NPQ, or L.C.
Press (SET) to confirm your selection in the R_delay Option and press (MENU) to return.

Press (MENU) to scroll down in the Setting Sub-Menu

>Multi

Press (SET) for setting the Multi Option

The Multi Option is used for multiple (automatically repeated) measurements of a selected parameter: Ft, QY, OJIP, NPQ, or L.C.

Press (MENU) to scroll down in the Setting Sub-Menu

>M_Type

Press (SET) for parameter

The M_Type Option allows selecting the repeatedly measured parameter: Ft, QY, OJIP, NPQ, or L.C.

Press (SET) to confirm your selection. Press (MENU) to return.

>R_Inter

Press (SET) for time interval

The R_Inter Option allows to set the time interval (in minutes) between repeated measurements.

Press (SET) to confirm your selection. Press (MENU) to return.

>R_Count

Press (SET) for count

The R_Count Option allows setting the number of repeated measurements.

Press (SET) to confirm your selection. Press (MENU) to return.

>Use RS

Press (SET) to confirm

The Use RS Option (Use Repeatedly Setting) serves to confirm the Repeat and R_Delay Options for each measurement performed within the Multi Option. Choose YES or NO.

>Return

Press (SET) to return to the Setting Sub-Menu

To be continued on the next page

14

Setting Sub-Menu - Part 3

Use the Setting Sub-Menu to set the light color, light intensity, number and frequency of measurements, date, time, or the sound mode.

>Sound

Press (SET) for the sound setting → Choose YES or NO for the device beeping

Press (MENU) to scroll down in the Setting Sub-Menu

>Time

Press (SET) for setting the time and date → Set year, month, day, hour, minute, and second in the Time Option. Press (SET) to confirm your selection. Press (MENU) to scroll down inside this Option.

Press (MENU) to scroll down in the Setting Sub-Menu

>Setting ← >Return

Press (SET) to return to the Main Menu

Press (MENU) to scroll down in the Main Menu

Press (MENU) to scroll in the Setting Sub-Menu (to return to >Bluetooth)

7. Bluetooth Pairing and Connecting

This Chapter applies to users of the **FluorPens with an incorporated Bluetooth** communication module.

7.A. General Information

What you will need first:

Before you set up the Bluetooth connection between the FluorPen and PC, make sure you have these components:

1. Bluetooth enabled FluorPen
FluorPen FP100max and **FluorPen FP100b** include built-in Bluetooth capabilities.

2. Bluetooth enabled PC

The PC with which you connect must have Bluetooth wireless technology, either built-in or through a Bluetooth card. Make sure that the PC's Bluetooth setting is "discoverable" (meaning that it shows up when other devices search for nearby Bluetooth connections). Consult the user guide for your PC or Bluetooth card to learn how to do this.

3. Bluetooth configuration software properly set up on PC

Before you can exchange files with your PC, you will need to set up the Bluetooth software that came with your PC, or your PC's Bluetooth card. This software varies by manufacturer. Please consult your PC's Bluetooth documentation for more information.

4. Bluetooth must be switched on visible on both devices

To pair the FluorPen with another Bluetooth device, such as a computer, you will need to ensure that Bluetooth is switched on visible on both devices.

7.B. Bluetooth Pairing

Step 1: Enabling Bluetooth in the FluorPen

Switch on the FluorPen (press and hold the SET key).

Scroll to the "Setting" menu (press the MENU key twice, then press the SET key once).

Select "BT_On" to enable Bluetooth (press the SET key).

* Keep in mind that the FluorPen turns off automatically after about 3 minutes of no action. Turning off the FluorPen ***always turns off Bluetooth****.*
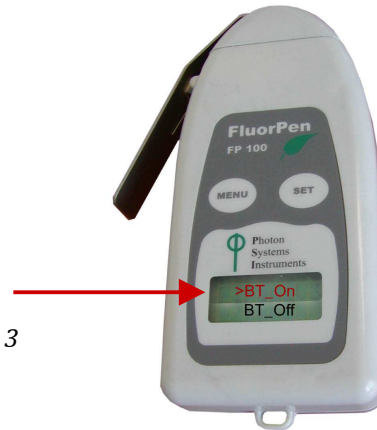
Step 2: Starting the Bluetooth Application on Your PC

Be aware that this description is a general example; some of the steps may be different on your PC.

Select: Start>All Programs>My Bluetooth Places.
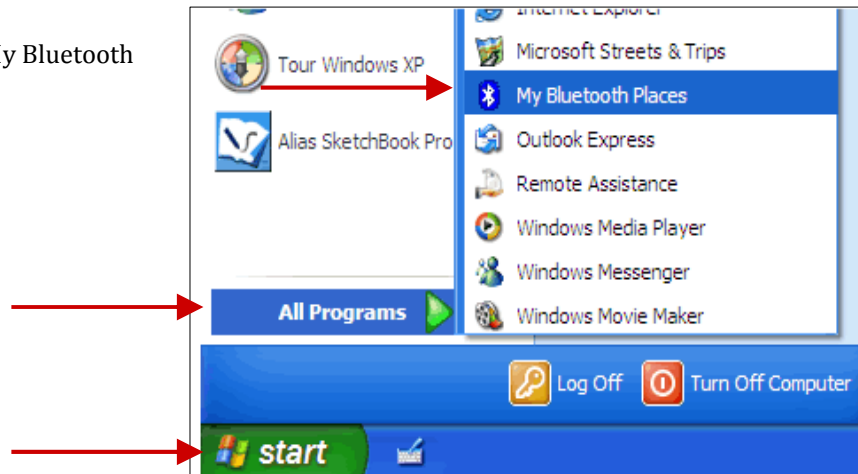
You may also start your Bluetooth application via the Control Panel:

Select: Start>Settings>Control Panel>Bluetooth Devices.

17

Step 3: Opening the Add Bluetooth Device Wizard

Select: "Add" to start the wizard.*

* Before starting to use the wizard, be sure that the FluorPen is in discoverable mode (see Step 1).
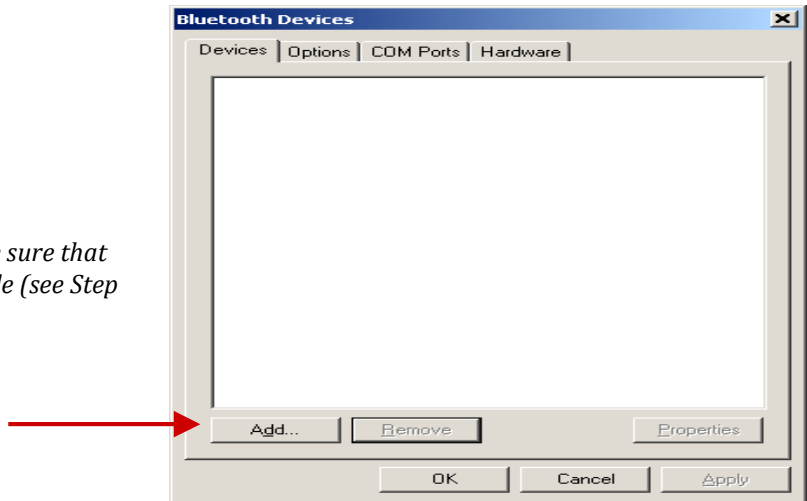
Step 4: Searching for a New Bluetooth Device

Mark the following box: "My device is set up and ready to be found".

Select: Next.

Step 5: Selecting the FluorPen

Select: Bluetooth FluorPen icon (FluorPen FluoroMeter PSI).*

Select: Next.

* Your display may show more Bluetooth Device icons.
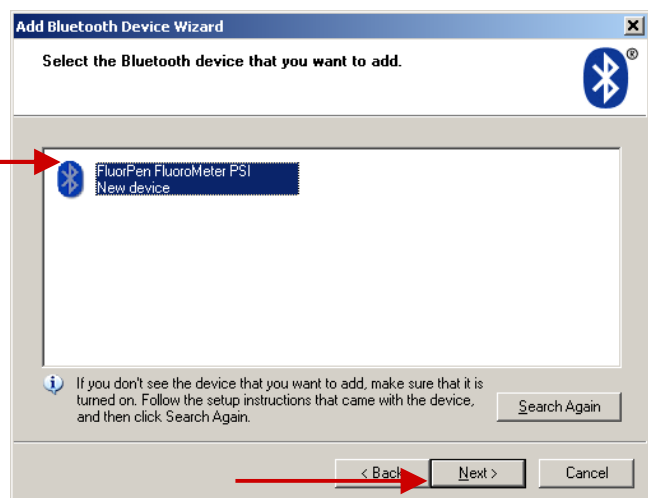
Step 6: Starting the Pairing Process

Your Bluetooth Pairing Passkey is:
          0000

Select: "Let me choose my own   passkey."

Enter: 0000 (four digits).

Select: Next.
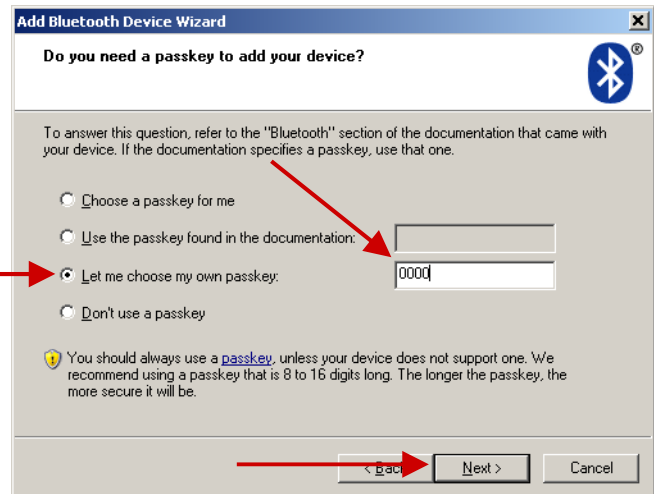
Step 7: Completing the FluorPen Pairing

Select: Finish.

7.C. Bluetooth Connection

Step 1: Installing the FluorPen Software

Install the FluorPen software to your PC. Use the **CD** that came **enclosed** with your FluorPen.*

* See Chapter 8 of this Operation Manual for complete information on FluorPen software.

Step 2: Enabling Bluetooth in the FluorPen

Switch on the FluorPen (press and hold the SET key).

Scroll to the "Setting menu" (press the MENU key twice, then press the SET key once).

Select "BT_On" to enable Bluetooth (press the SET key).*

** Keep in mind that the FluorPen turns off automatically after about 3 minutes of no action. Turning off the FluorPen **always turns off Bluetooth**.*

Step 3: Starting Connection

Start the FluorPen software on your PC.

Select: Setup>Device ID (Ctrl+I).

If properly connected, the message "Device: FluorPen" appears on the bottom part of the screen.
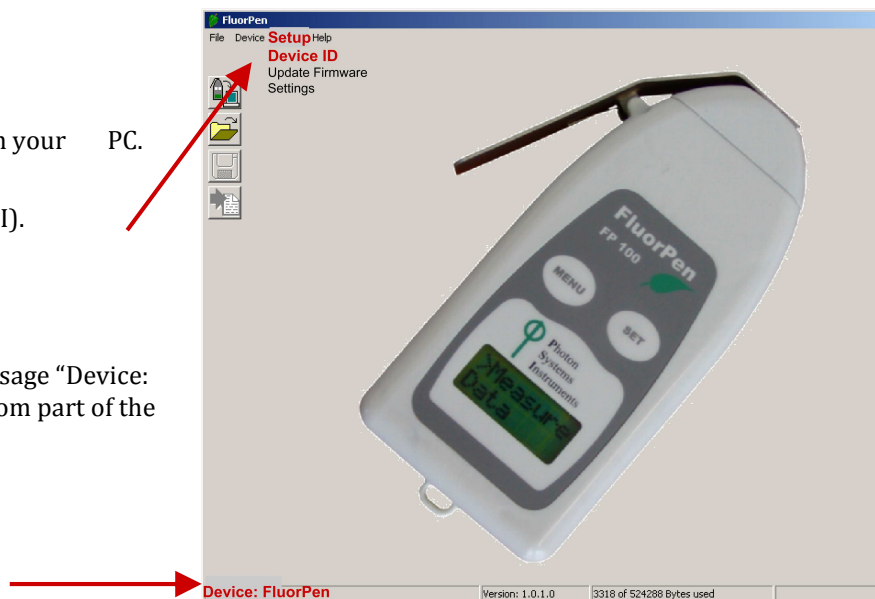
Step 4: Registering the FluorPen Software

Select: Help>Register.

Enter your serial (registration) number.*

Select: OK.
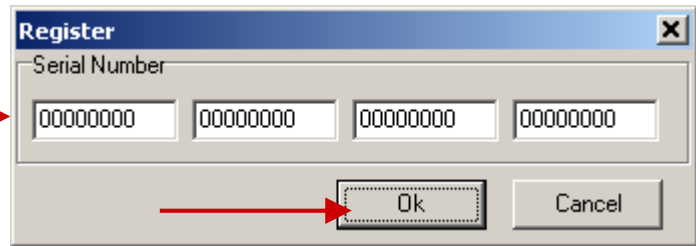
** You will find your serial (registration)
    number in the file **SN.txt on the enclosed CD**.*

Important Note: How to Reconnect Bluetooth

Disconnection can occur, either when the Bluetooth feature has been turned off in one or both of the devices, or when the units move outside their operating range.

If the devices have been turned off, simply turn them on and enable Bluetooth in the FluorPen again.

If the FluorPen has been moved outside the Bluetooth operational range, bringing it back into range within 90 seconds will allow it to reconnect automatically. If more time elapses, simply turn the FluorPen on and enable Bluetooth again.

8. FluorPen Software

This Chapter applies to users of the **FluorPen FP 100-MAX** and to users of the **FluorPen FP 100-B, FP 100-S, and FP 100-U**.

8.A. Starting up

Starting up:

1. Switch on the computer.

2. Switch on the FluorPen and enable Bluetooth.*

3. Make sure that your PC and the FluorPen are properly paired.*

4. For FluorPens supplied with the USB communication module, use provided USB cable to connect your device to a PC.

5. Start the FluorPen program.

Points 2 and 3 apply only to users of the FluorPen FP 100-MAX and FluorPen FP 100-B. See Chapter 7 of this Operation Manual for complete information on Bluetooth pairing and connecting.

Connecting:

Select: Setup>Device ID (Ctrl+I).

If properly connected, the message "Device: FluorPen" appears in the bottom part of the screen.

8.B. Menu and Icon Explanation

Menu: File

Load	Loads previously saved data files.
Save	Saves data to hard disc.
Export	Exports data in .txt format.
Close	Closes the current experiment.
Close All	Closes all running experiments.
Exit	Exits the program.
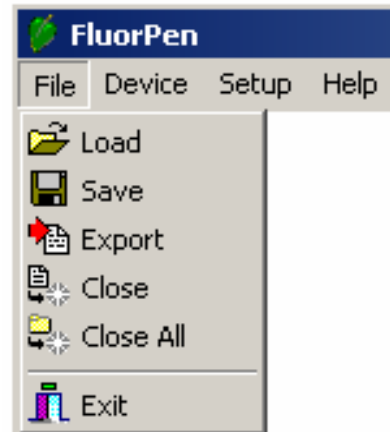
Menu: Device

Download	Downloads data from the FluorPen to your PC.
Erase Memory	Erases data from the FluorPen memory.
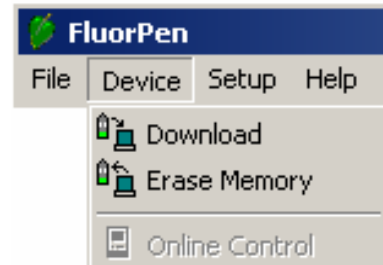
Menu: Setup

Device ID	Detects the Bluetooth connected device.
Update Firmware	Used for software updates.*
Settings	Used for modification of the program settings.**
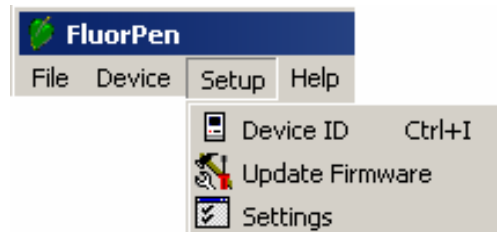
For more information on software updating, see Chapter 8D of this Operation Manual.

*** See more information on the next page.*

Menu: Help

About	Offers basic information about the program.
Register	Used for the FluorPen software registration.*
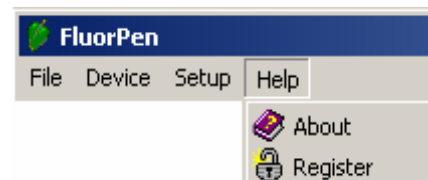
* *See Chapter 7C of this Operation Manual for more information on FluorPen software registration.*

Menu: Settings

After Download - Memory Erase

If the box is checked, the FluorPen memory is erased after each data download.

Data - Inverted

If the box is checked, the polarity of data is inverted, e.g., multiplied by -1.*

.

Data – Add to Opened

If the box is checked, the downloaded data are added to that of the current opened experiment.
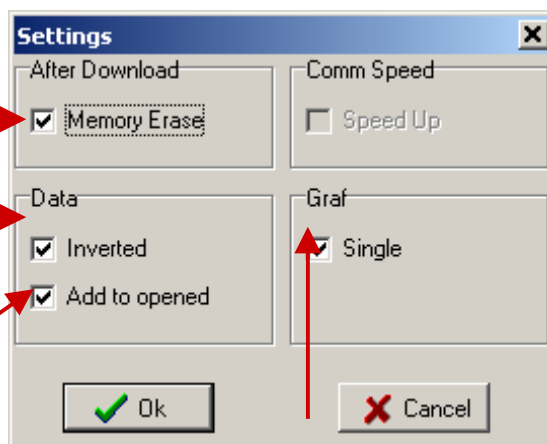
Graf - Single

If the box is checked, all measured data are visualized in one graph, i.e., the value of each new measurement is added to the currently used graph window.

If the box is not checked, a new graph is opened for every new measurement.

* This feature can be helpful for a certain type of experiment when the measured data are undesirably interpreted as negative values.

Icon Explanation:

	Download	Downloads data from the FluorPen to PC.
	Load	Loads (opens) previously saved data files.
	Save	Saves data to hard disc.
	Export	Exports data in .txt format.

8.C. Example of Data Transfer and Visualization

Step 1: Do a measurement with your FluorPen.
(Here, we did an OJIP measurement.)

Step 2: Click the "Download" icon or select Device>Download.

Step 3: The Data table appears.

Callouts on the figure: File name · Time of experiment · Measured parameter · Save experiment · Measured and calculated values · Space for written notes · Switch to graphic visualization of the experiment · Experiment description

T:\Final\FluorPen\Test_Data\data.dat

Time	6:26:3 2.1 2007	6:26:8 2.1 2007	6:26:32 2.1 2007	6:27:25 2.1 2007	6:4_:_9 2.1 2007
ID	QY	OJIP	Ft	QY	OJIP

QY: 0.64
Fo Backgr 1
Fo Flash 351
Fm Backgr 1
Fm Flash 964

OJIP (Arabidopsis A):
Fo 618
Fj 1422
Fi 2149
Fm 2423
Fv 1805
Vj 0.445
Vi 0.848
Fm/Fo 3.921
Fv/Fo 2.921
Fv/Fm 0.745
Mo 0.911
Area 4786890
Sm 2652.017
Ss 0.489
N 5422.780
Phi_Po 0.745
Psi_o 0.555
Phi_Eo 0.413
Phi_Do 0.255
Phi_Pav 2857.311
ABS/RC 2.745
TRo/RC 2.045
ETo/RC 1.134
DIo/RC 0.700

Ft: 355
Backgr 1
Flash 356

QY: 0.71
Fo Backgr 1
Fo Flash 275
Fm Backgr 1
Fm Flash 942

OJIP (Arabidopsis B):
Fo 646
Fj 1491
Fi 2438
Fm 2639
Fv 1993
Vj 0.424
Vi 0.899
Fm/Fo 4.085
Fv/Fo 3.085
Fv/Fm 0.755
Mo 0.831
Area 5229888
Sm 2624.129
Ss 0.510
N 5142.671
Phi_Po 0.755
Psi_o 0.576
Phi_Eo 0.435
Phi_Do 0.245
Phi_Pav 2908.289
ABS/RC 2.595
TRo/RC 1.960
ETo/RC 1.129
DIo/RC 0.635

Description: Arabidopsis A · Arabidopsis B

Data | Notes | Graph:6:26:8 2.1 2007

Device: FluorPen | Version: 1.0.1.0 | 0 of 524288 Bytes used

Step 4: To visualize measurement in the graphic mode, click the "Graph" field in the bottom bar.

Step 5: The colored Graph of measured data appears.

Selection of data corresponds to their description

Choose logarithmic or non-logarithmic graph form
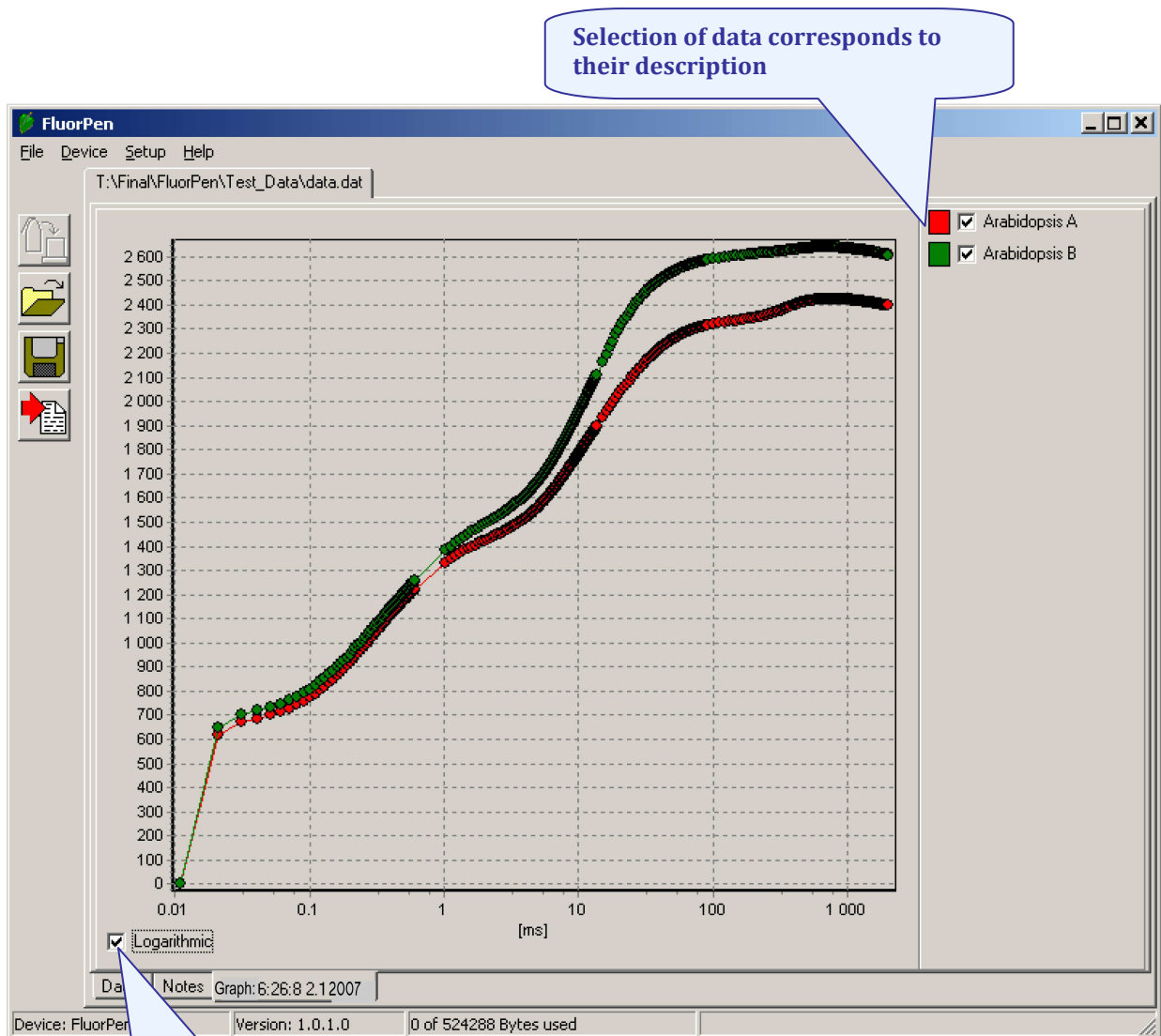
8.D. Explanation of OJIP Parameters

The FluorPen also offers the possibility to capture rapid fluorescence transient – OJIP, which occurs during exposure of plants to high irradiance. The FluorPen software enables data downloading to a personal computer and subsequent OJIP analysis. The OJIP protocol includes the following extracted and technical parameters*:

Formula Abbreviation	Formula Explanation
Bckg	Background
F_0	$F_0 = F_{50\mu s}$, fluorescence intensity at 50 μs
F_J	F_J = fluorescence intensity at J-step (at 2 ms)
F_i	F_i = fluorescence intensity at i-step (at 60 ms)
F_M	F_M = maximal fluorescence intensity
F_V	$F_V = F_M - F_0$ (maximal variable fluorescence)
V_J	$V_J = (F_J - F_0) / (F_M - F_0)$
V_i	$V_i = (F_i - F_0) / (F_M - F_0)$
F_M / F_0	
F_V / F_0	
Fv / F_M	
M_0 or $(dV/dt)_0$	$M_0 = TR_0 / RC - ET_0 / RC = 4 (F_{300} - F_0) / (F_M - F_0)$
Area	Area between fluorescence curve and F_M (background subtracted)
Fix Area	Area below the fluorescence curve between $F_{40\mu s}$ and F_{1s} (background subtracted)
S_M	$S_M = Area / (F_M - F_0)$ (multiple turn-over)
S_S	S_S = the smallest S_M turn-over (single turn-over)
N	$N = S_M \cdot M_0 \cdot (1 / V_J)$ turn-over number Q_A
Phi_P$_0$	$Phi\_P_0 = 1 – (F_0 / F_M)$ (or F_V / F_M)
Psi_$_0$	$Psi\_0 = 1 - V_J$
Phi_E$_0$	$Phi\_E_0 = (1 – (F_0 / F_M)) \cdot Psi\_0$
Phi_D$_0$	$Phi\_D_0 = 1 – Phi\_P_0 – (F_0 / F_M)$
Phi_Pav	$Phi\_Pav = Phi\_P_0 (S_M / t_{FM})$ t_{FM} = time to reach F_M (in ms)
ABS / RC	$ABS / RC = M_0 \cdot (1 / V_J) \cdot (1 / Phi\_P_0)$
TR_0 / RC	$TR_0 / RC = M_0 \cdot (1 / V_J)$
ET_0 / RC	$ET_0 / RC = M_0 \cdot (1 / V_J) \cdot Psi\_0$
DI_0 / RC	$DI_0 / RC = (ABS / RC) – (TR_0 / RC)$

* Formulas Derived From:
R.J. Strasser, A. Srivastava and M. Tsimilli-Michael (2000): The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Probing Photosynthesis: Mechanism, Regulation and Adaptation (M. Yunus, U. Pathre and P. Mohanty, eds.), Taylor and Francis, UK, Chapter 25, pp 445-483.

8.E. Non-Photochemical Quenching (NPQ) Protocol

The NPQ protocol is the most typically used measuring approach to quantify photochemical and non-photochemical quenching. The measurement should be performed with a dark-adapted sample. Thereby, it may not be appropriate under field conditions.

The NPQ protocol starts by giving a measuring light to acquire minimal level of fluorescence *Fo.* A short saturating flash of light is then applied to reduce the plastoquinone pool and measure maximum fluorescence in the dark adapted state, *Fm*. After a short dark relaxation, the sample is exposed to actinic irradiance for tens to hundreds of seconds to elicit a transient of the Kautsky effect. Moreover, a sequence of saturating flashes is applied on top of the actinic light to probe the non-photochemical quenching *NPQ* and effective quantum yield of photosynthesis *QY* in light adapted state. After exposure to continuous illumination, the relaxation of non-photochemical quenching is determined by means of saturating pulses applied in dark.

Two NPQ protocols, NPQ1 and NPQ2 are predefined. The protocols differ in the duration of the light exposure and the dark recovery phase, in the number and interval between pulses. See table below.

	Phase	Duration	# of pulses	1st pulse	Pulse interval
NPQ1	Light	60s	5	7s	**12s**
	Dark recovery	88s	3	11s	26s
NPQ2	Light	200s	10	10s	20s
	Dark recovery	390s	7	20s	60s

Abbreviation	Explanation
Fo	minimum fluorescence in dark-adapted state
Fm	maximum fluorescence in dark-adapted state, measured during the first saturation flash after dark adaptation
Fp	fluorescence in the peak of fast Kautsky induction
Fm_L, Lss, D, Dss[1]	maximum fluorescence
QYmax[2]	maximum quantum yield of PSII in dark-adapted state - Fv/Fm
QY_L, Lss, D, Dss[1,3]	effective quantum yield of PSII
NPQ_L, Lss, D, Dss[1,4]	non-photochemical chlorophyll fluorescence quenching
Qp_L, Lss, D, Dss[1,5]	coefficient of photochemical quenching, an estimate of open PSII reaction centers

[1] *L* - indicates light adapted parameters; *D* - refers to dark recovery phase after switching of the actinic illumination; *n* - represents a sequential number of light phase; *ss* - steady state
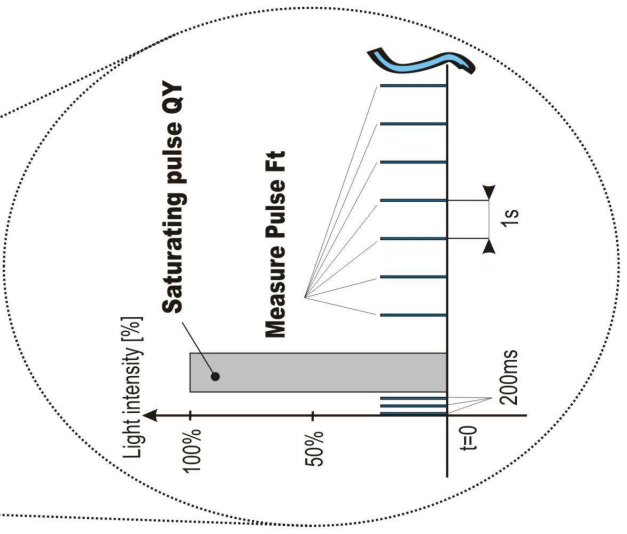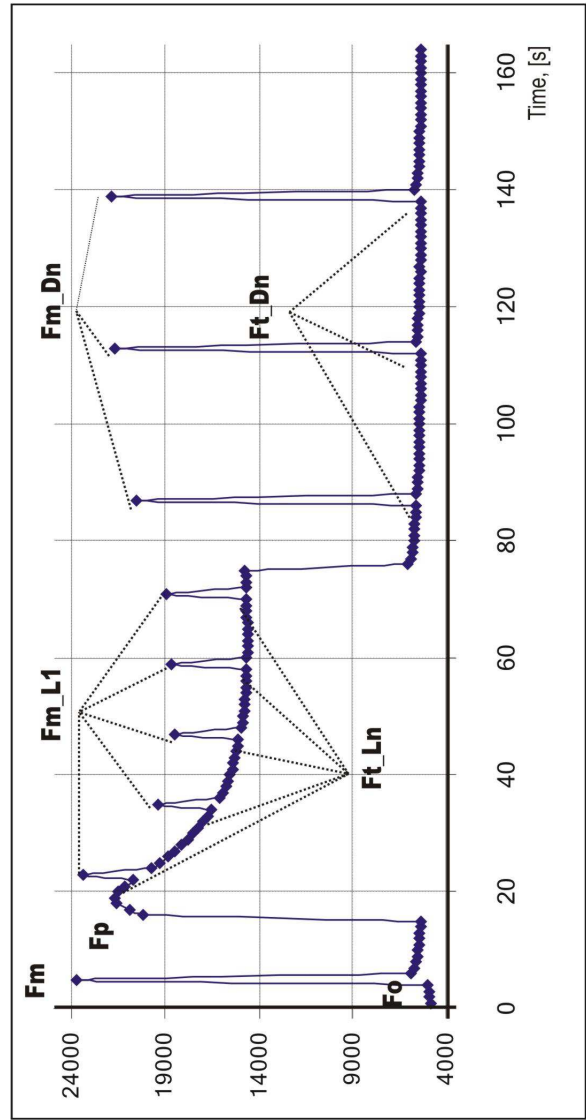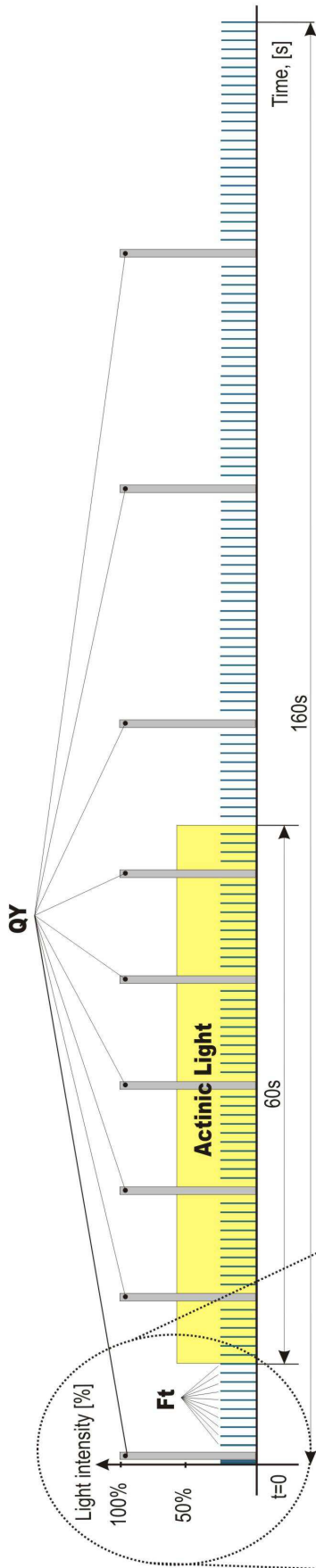
[2] Calculated as *(Fm – Fo) / Fm*

[3] Calculated as *(Fm_Ln – Ft_Ln) / Fm_Ln* or of corresponding steady state or dark recovery parameters

[4] Calculated as *(Fm – Fm_Ln) / Fm_Ln* or of corresponding ss, Dn or Dss parameters

[5] Calculated as *(Fm_Ln – Ft_Ln) / (Fm_Ln – Fo_Ln)* or of corresponding ss, Dn or Dss parameters

NPQ Protocol

29

8.F. Light Curve (LC) Protocol

The protocol called Light Curve (LC) was designed to acquire parameters for construction of Light Response Curve relating the rate of photosynthesis to photon flux density. The method is based on successive measurements of the sample exposed to a stepwise increase of light intensity. The effective quantum yields of photosynthesis are determined under various light intensities of continuous illumination. Measurement is based on pulse modulated fluorometry (PAM).

Several LC protocols are predefined in FP. These differ in number and duration of individual light phases and light intensities.

	# of phases	Phase duration	Light intensities, $[\mu mol\ m^{-2}\ s^{-1}]$
LC1	6	**60s**	10; 20; 50; 100; 300; 500
LC2	5	30s	100; 200; 300; 500; 1000
LC3	7	60s	10; 20; 50; 100; 300; 500; 1000

The protocol includes following measured and calculated parameters:

Abbreviation	Explanation
Fo	minimum fluorescence in dark-adapted state
Fm	maximum fluorescence in dark-adapted state
Fm_Ln[‡]	maximum fluorescence in light adaptation state
Ft_Ln[‡]	instantaneous fluorescence during light adaptation
QYmax[*]	maximum quantum yield of PSII in dark-adapted state - Fv/Fm
QY_Ln[‡**]	instantaneous PSII quantum yield induced in light

[‡] n represents a sequential number of light phase

[*] Calculated as (Fm – Fo) / Fm

[**] Calculated as (Fm_Lx – Ft_Lx) / Fm_Lx

Light Curve 1 Protocol

500 μmol(photon)/m²

300 μmol(photon)/m²

100 μmol(photon)/m²

50 μmol(photon)/m²

20 μmol(photon)/m²

t[sec]

360s

QY

Ft

Light intensity [%]

100%

50%

t=0

10sec 10sec 10sec 10sec 10sec 10sec
60ses

Actinic Light Intensity: 10 μmol(photon)/m²

Super Pulse

Actinic Light

Measure Pulse Ft

Fm

Fo

100%

50%

t=0

10sec 10sec 10sec 10sec 10sec 10sec
60sec

31
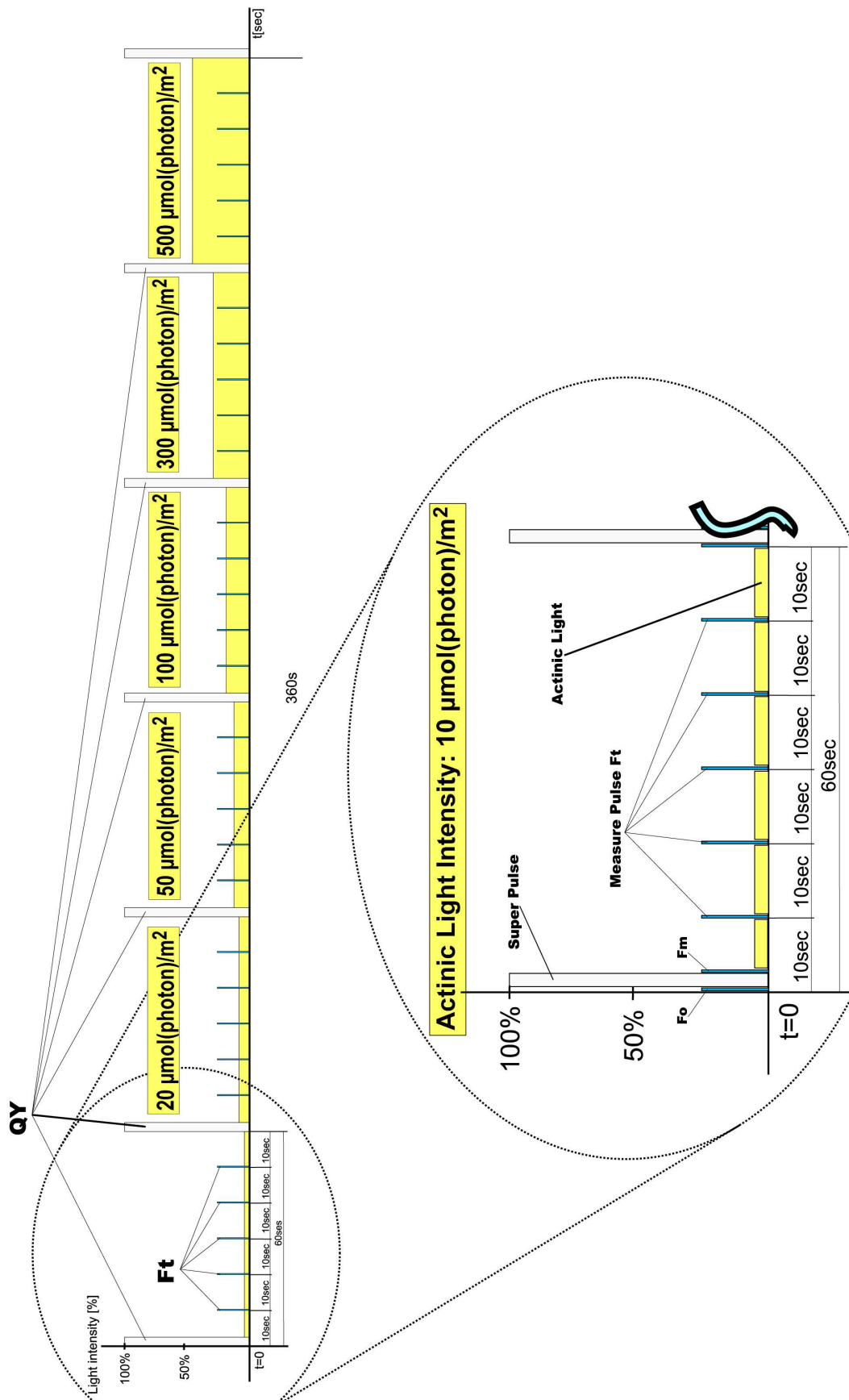
Light Curve 2 Protocol
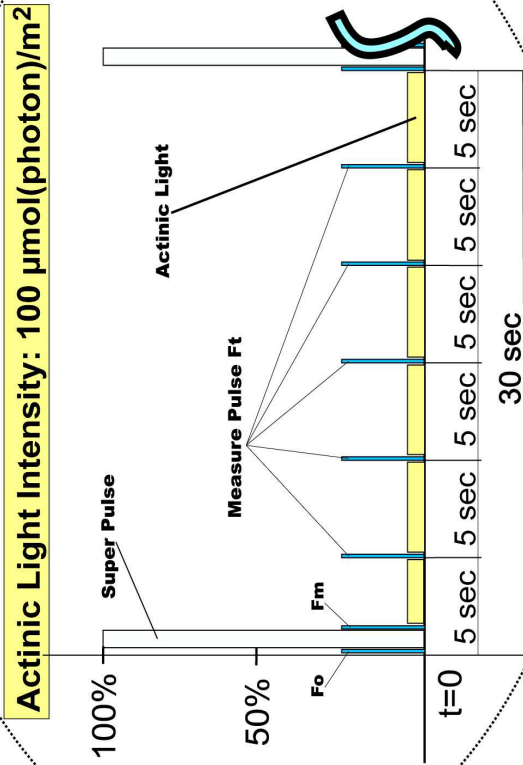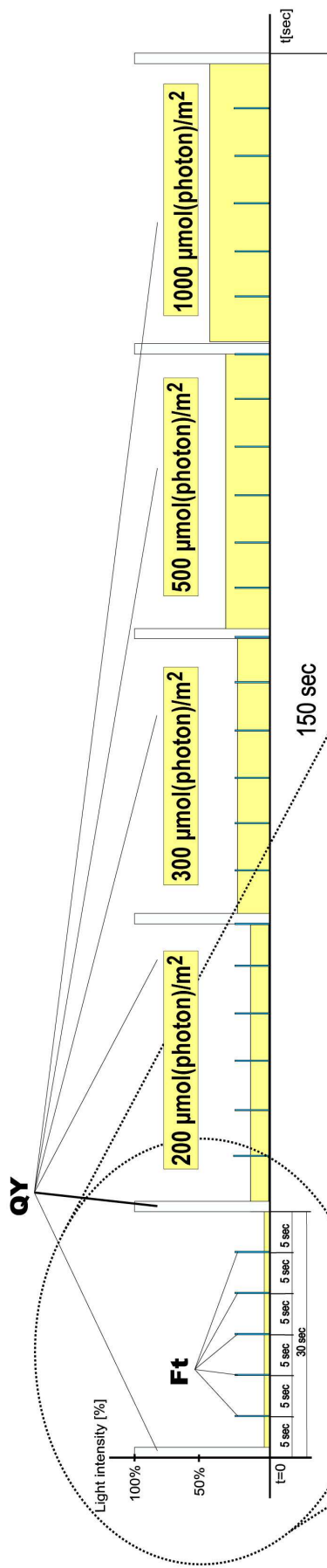
8.G. Software Update

> **Very important!**
>
> **The FluorPen memory is erased during the software update!**
>
> **Before starting any software update, export all your data from the FluorPen memory into your computer!**

Step 1: Starting Update

Select: Setup>Update Firmware

Step 2: Selecting .bxn File

Find: Binary file (with the extension .bxn)

Select: Open
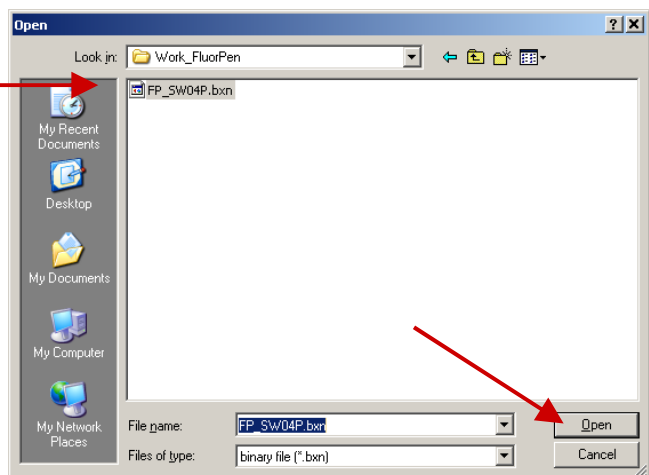
Step 3: Finishing Upload

Select: "OK" to start uploading of the update.
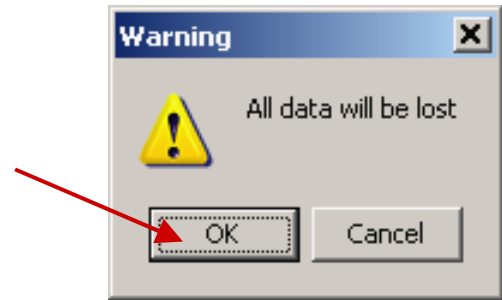
The bottom bar indicates the upload progress.
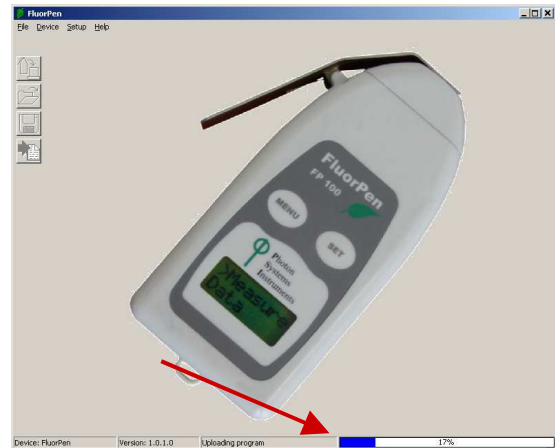
Press: "OK" to finish upload.
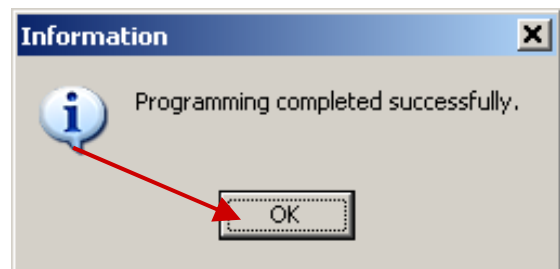
9. GPS Module

> **IMPORTANT INFORMATION:**
>
> For proper GPS reading, the **time in your FluorPen and in your computer must be synchronized!** Preset time and time zone must correspond to GPS time (time zone) in your location.

9.A. GPS Module Description

1. GPS Status Indicator
2. Memory Storage Indication

3. Power Key
4. Battery Status Indication

5. Power Charge Jack
6. USB Download

1. GPS Status Indicator

Green LED On/Off (On 1 sec/OFF 2 sec): Acquiring satellites.

Green LED On/Off (flashing 2 sec/2 sec): Position fixed.

2. Memory Storage Indicator

Red LED On: Memory storage full.

Orange LED On: Memory storage full + GPS unfixed.

Orange LED On/Off (flashing 2 sec/2 sec): Memory storage full + GPS fixed.

3. Power Key

Turn On: Press the key once and hold it for 3 sec.

Turn Off: Press the key twice (hold the second press for 3 sec).

4. Battery Status Indication

Red LED On/Off (flashing 1 sec/2 sec): Battery low (turns off after 5 minutes).

Red LED On: Battery charging.

Red LED Off: Battery full or not charging.

Note:

GPS Module is equipped with Lithium-ion rechargeable battery that lasts up to 24 hours of use. Recharging the battery takes about 4 hours.

9.B. First Time Installation of the GPS Module

Step 1: Enabling GPS Module in the FluorPen Software

Select: Setup>Settings

Select: Settings>GPS visible

Step 2: Accepting GPS Setting

Close and restart the FluorPen program to accept GPS setting.

9.C. GPS / FluorPen Operation

Step 1: Time Synchronization

Synchronize the FluorPen time with the time of your PC. Time must be set correctly witch respect to your time zone.

Step 2: GPS Positioning

Switch the GPS module on and wait until the GPS position is fixed (GPS green LED indicator flashes 1s ON and 2s OFF while tracking the position).

Step 3: Operation

Be aware that while performing field experiments, the FluorPen and the GPS module must be kept close to each other.

9.D.   Data Download

Step 1: Enabling Communication

Switch on the computer, FluorPen, and GPS module and set your computer to FluorPen communication (enable Bluetooth or connect to serial or USB port).

Step 2: Downloading FluorPen Data

Start: FluorPen program.

Connect: FluorPen device.

Download: Measured data from the FluorPen to your PC.
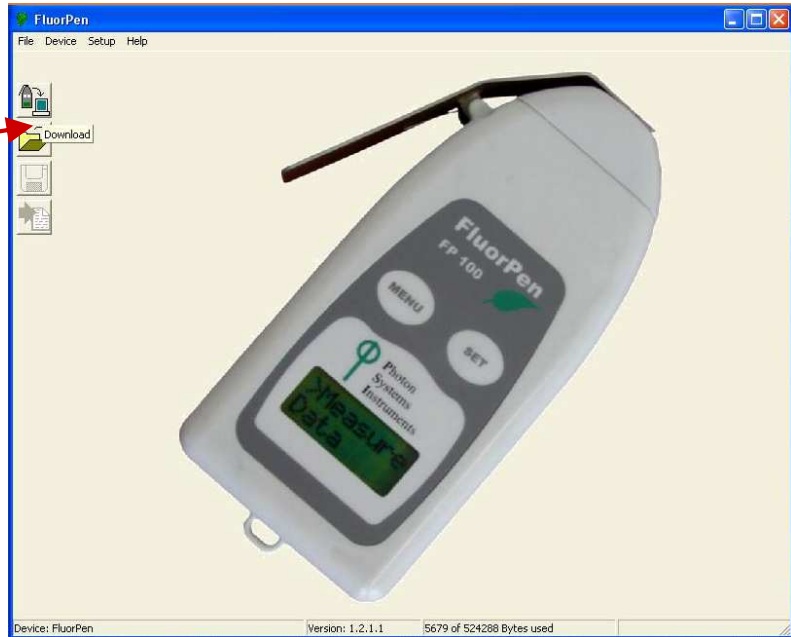
Be aware that no GPS coordinates are visible at this moment.
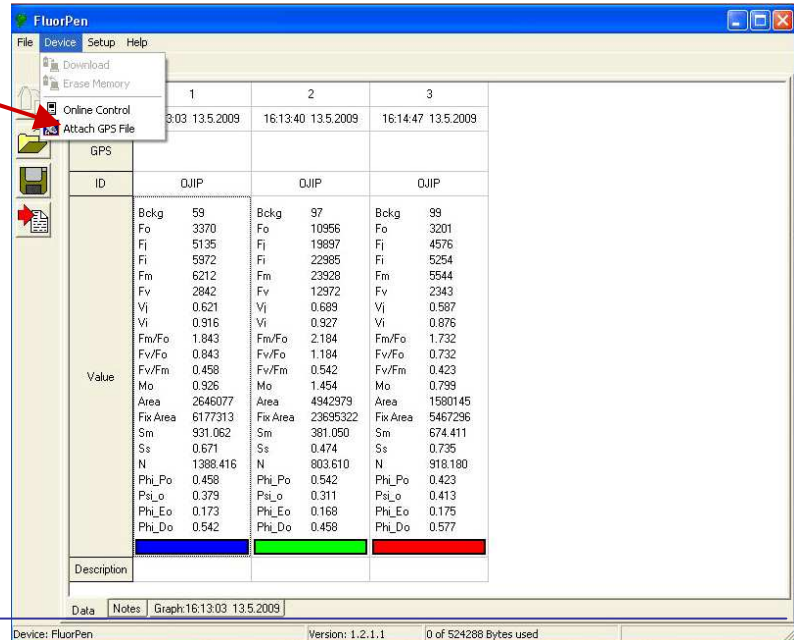
Step 3: Connecting GPS Module

Connect the GPS Module to your PC. Communication is set properly if the hardware is recognized by your PC.
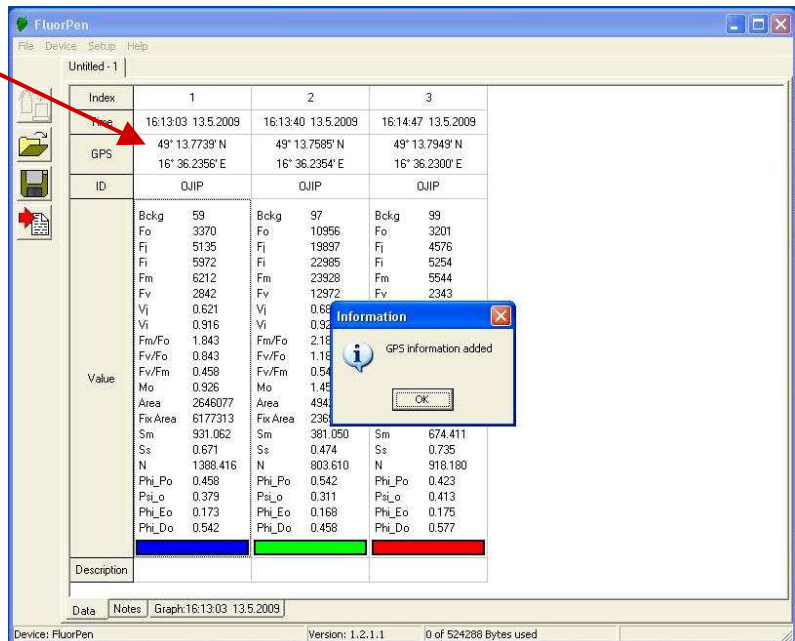
Step 4: Downloading GPS Data

Select:

Device>Attach GPS file

to download data from the GPS module.

Step 5: Completing the Download

Successfully downloaded GPS coordinates paired with FluorPen data.

10. Statement of Limited Warranty

- This Limited Warranty applies only to the FluorPen and its accessories (excluding any batteries). It is valid one year from the date of shipment.

- If at any time within this warranty period the instrument does not function as warranted, return it and PSI will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. PSI is responsible for shipping and insurance on return of the instrument to the customer.

- No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by PSI; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by PSI.

- The warranty is return-to-base only, and does not include on-site repair charges such as labor, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.

- PSI repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.

- PSI will keep spare parts or their adequate substitutes for a period of at least five years.

- Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.

- PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.

- *Wear & Tear Items* (such as sealing, tubing, padding, etc.) are excluded from this warranty. The term *Wear & Tear* denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.

*For customer support, please write to: **support@psi.cz***

Copyright © Photon Systems Instruments, 2013-01